Production and purification of frutalin, a potential cancer marker, in *Escherichia coli*

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Introduction

Frutalin, the α-D-galactose-binding lectin isolated from *Artocarpus incisa* seeds (breadfruit seeds), is a protein similar to jacalin, the well studied galactose-binding lectin isolated from the *Artocarpus integrifolia* seeds (jackfruit seeds). Like jacalin, frutalin binds specifically D-galactose but has a more marked biological activity, as it is shown by its higher haemagglutination activity (Campana *et al.* 2002). Active frutalin is a tetrameric protein which monomers are made of one α chain and one β chain bound by noncovalent linkages and contains a binding site for D-galactose (Moreira *et al.* 1998). This lectin is thought to be synthesized as a pre-pro-lectin, consisting of a signal sequence, a pro-peptide, a β-chain, a linker peptide and a α-chain. In mature frutalin, the signal sequence and the pro-peptide are moved through post- and/or co-translational modifications, and the linker is also excised to separate α and β chains.
Frutalin can be potentially use as a cancer diagnostic tool due to its ability to bind galactose complexes of cancer cell surfaces. This application will require large amounts of this lectin with the highest level of purity. The heterologous expression of frutalin in microorganisms may simply improve its availability. *Escherichia coli* was chosen as host strain for frutalin expression because it is a well studied expression system and offers a mean of rapid and economical production of recombinant proteins. These advantages coupled with a wealth of biochemical and genetic knowledge make *E. coli* one of the most popular systems for protein production (Jana and Deb, 2005; Kim *et al.* 2007). The aim of this work is to maximize the expression and purification of soluble biologically active recombinant frutalin in *E. coli*.

Material and methods

Frutalin codifying sequence was cloned into the multiple cloning site of the pET-25b(+) expression vector (Novagen). Recombinant frutalin was expressed with its native C-terminal in the bacterial strain *Escherichia coli* BL21-CodonPlus(DE3)-RIPL (Stratagene). LB medium, supplemented with 100 µg/ml of ampicillin and 50 µg/ml of chloramphenicol, was used for *E. coli* recombinant strains selection, maintenance and expression.
Frutalin expression was optimized after recombinant *E. coli* induction with different concentrations of IPTG (0.1 and 1 mM), incubated at different temperatures (22 and 37ºC) for different time periods (3 and 20 h). The *E. coli* cell-free extracts were obtained after cell lyses by sonication and clarification by centrifugation. Recombinant frutalin was detected in the *E. coli* soluble fractions by denaturing SDS-PAGE electrophoresis and Western blot analysis. Soluble recombinant frutalin was quantified on scanned gels using the Quantity One software (Bio-Rad). The effect of the experimented variables on the soluble expression of recombinant frutalin was assessed by implementing a factorial experimental design.
Size exclusion chromatography, with a Sephacryl® S-100 HR column and a FPLC system (Pharmacia Biotechnology), was performed as a preliminary step to purify recombinant frutalin from the *E. coli* cell-free extracts. The presence of recombinant

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lectin in the pooled fractions was made by checking its agglutination activity with rabbit erythrocytes. Fractions purity was evalueted by SDS-PAGE followed by silver nitrate staining.

Results and discussion

A single band of about 16-17 KDa, corresponding to recombinant frutalin, was detected in the soluble fractions of the *E. coli* extracts, and not detected in the extracts of the negative control (*E. coli* bearing the empty pET-25b(+) vector), by both SDS-PAGE and Western Blot analyses. This molecular weigth has a good agreement with the calculated molecular weight of the amino-acid residues of β and α chain conneted throught the linker of the cloned frutalin sequence (17.1 KDa). That fact may lead to the possibility that recombinant frutalin does not undergo any proteolytic cleavage in *E. coli*, as occurred with jacalin produced in *E. coli* (Sahasrabuddhe *et al.* 2004).

The factorial experimental design resulted in a third order interaction model demonstrating that the maximum soluble recombinant frutalin was obtained at 22ºC and 20 h of induction with 1 mM IPTG (12.6 mg/l). Temperature and concentration of IPTG were the main factors that most contribute to the soluble frutalin expression. The interaction between concentration of IPTG and induction length was the most significant interaction factor.

Haemagglutination activity in the *E. coli* extracts was only detected after a dilution factor of two or four times, suggesting the presence of agglutination inhibitory components. Size exclusion chromatography resulted in 50 ml of pooled fractions with haemagglutination activity per litre of *E. coli* culture. Several of these fractions presented a high level of purity, showing that most of the *E. coli* proteins contaminants were removed, nevertheless further purification and concentration steps will be needed, such as affinity or ion-exchange chromatography. The fact that recombinant frutalin exhibits haemagglutination activity, inhibited by D-galactose, in conjunction with the elution time in the Sephacryl column, which is higher than the BSA (66 KDa) elution time, suggests that it may have the tetrameric native structure (Sahasrabuddhe *et al.* 2004).

Conclusion

In this work, biologically active recombinant frutalin was successfully produced in *E. coli* and partially purified using size exclusion chromatography.

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